CHROMOSOMES OF *TAKHTAJANIA*, OTHER WINTERACEAE, AND CANELLACEAE: PHYLOGENETIC IMPLICATIONS[1]

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ABSTRACT

The somatic chromosome number of *Takhtajania perrieri*, the only relict representative of Winteraceae on Madagascar, is documented as $2n = 36$. The possibly paleotetraploid karyotype appears "diploidized." Chromosome size and behavior, as well as structure of interphase nuclei, correspond to the prochromosome type, widespread among the Magnoliid assemblage. Chromosome numbers available for all other Winteraceae and the related Canellaceae (Winterales) are critically compiled, compared, and interpreted. In light of the postulated molecular phylogeny of the two families, it appears that several successive phases of polyploidization and parallel descending dysploid series coupled with much extinction can explain the karyological features and distribution of the few surviving taxa.

Key words: Canellaceae, chromosome number, diploidization, evolution, paleopolyploidy, *Takhtajania*, Winteraceae.

Takhtajania perrieri (Capuron) Baranova & J.-F. Leroy was discovered in 1909 by Perrier de la Bâthie in the Massif du Manongarivo of northwestern Madagascar, where the population is now extirpated. Only in 1963 was it described as *Bubbia perrieri* by Capuron. After 85 years, its rediscovery in another locality of northwestern Madagascar, 150 km to the east, in the Anjanaharibe-Sud Special Reserve (Schatz et al., 1998) has made it possible to collect material for a first karyological study of this fascinating relict plant. After several failures with fixations of untreated roots and flower buds (smallest about 2 mm diam.), already too advanced for the study of microsporocyte meioses or pollen mitoses, preliminary results were finally obtained on mitotic chromosome number and morphology, as well as interphase nuclear structure from pretreated root tips.

This breakthrough has stimulated a critical compilation of all chromosome counts (and additional karyological observations) published so far on the families of Winteraceae and Canellaceae. That these families are related and somewhat isolated as Winterales within the Magnoliid assemblage (sister to Piperales sensu lato) has been well supported recently by plastid, mitochondrial, and nuclear DNA sequences (Soltis et al., 1999; Qiu et al., 1999). The aim of the present report is to summarize the still quite incomplete karyological data on all taxa of Winterales (Table 1) and to compare them with available information from DNA data as well as other evidence (see Vink, 1970, 1985, 1993; Kubitzki, 1993). Thus, we hope to contribute to a more unified picture of the evolution and phylogenetic history of the two Winterales families.

MATERIALS AND METHODS

The material used for our study was collected by C. Birkinshaw, 7–11 September 1997, at the Anjanaharibe-Sud Special Reserve, 14°45′S, 49°29′E, at an altitude of 1100–1300 m. Juvenile plants less than 20 cm tall but exhibiting adult leaf morphology were observed growing adjacent to reproductive adults. Ten of these were transplanted into containers and transported to Antananarivo. This population is represented by the fruiting voucher *C. Birkinshaw et al. 483.* At the time that root tips were fixed for the present study (June 1998) only two of the ten plants remained alive; both of these have subsequently died.

Root tips were pretreated in a 0.05% solution of colchicine for 3.5 hours at room temperature, fixed in a 1:3 mixture of glacial acetic acid and 96% ethanol, and stored in a deep-freezer until required.

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Table 1. Critical survey of chromosome number records available for Winteraceae and Canellaceae. First column with currently accepted and valid names of taxa, second column with names used in the original karyological publications.

Taxon	Published name	Provenance	$n/2n$	Reference
Winteraceae				
Takhtajania *perrieri* (Capuron) Baranova & J.-F. Leroy	same	Madagascar, Anjanaharibe-Sud	$2n = 36$	this publication
Tasmannia *insipida* R. Br. ex DC.	*Drimys insipida* (R. Br.) Pilger	Australia, N. S. Wales	$n = 13$	Hotchkiss (1955)
T. insipida R. Br. ex DC.	*Drimys insipida* (R. Br.) Pilger	Australia, N. S. Wales	$n = 13$, $2n = 26$	Ehrendorfer et al. (1968)
T. insipida R. Br. ex DC.	*Drimys insipida* (R. Br.) Pilger	Australia, S. Queensland	$n = 13$, $2n = 26$	Ehrendorfer et al. (1968)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	Australia, N. S. Wales	$2n = 13$	Hotchkiss (1955)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	? Australia	$2n = 26$	Smith-White in Darlington & Wylie (1955)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	? cult.	$2n = 28$[1]	Gulline in Darlington & Wylie (1955)
T. lanceolata (Poir.) A. C. Smith	*Drimys aromatica*	? cult.	$2n = 38$[2]	E K. Janaki Ammal in Darlington & Wylie (1955)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	? cult.	$2n = 26$	R. Gankin in Stebbins (1958)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	cult.	$2n = 26$	Raven & Kyhos (1965)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	Australia, N. S. Wales	$n = 13$, $2n = 26$	Ehrendorfer et al. (1968)
T. lanceolata (Poir.) A. C. Smith	*Drimys lanceolata* (Poir.) Baill.	cult.	$2n = 26$	Ratter & Milne (1973)
T. piperita (Hook f.) Miers "entity 25. *heteromera*" (Vink, 1970)	*Drimys hatamensis* Becc.	Papua-New Guinea, Mt. Wilhelm	$2n = 28$[1]	Borgmann (1964)
T. piperita (Hook f.) Miers "entity 32. *cordata*" (Vink, 1970)	*Drimys rosea*	Papua-New Guinea, Mt. Wilhelm	$2n = 28$[1]	Borgmann (1964)
T. piperita (Hook f.) Miers "entity 38. *membranea*" (Vink, 1970)	*Drimys membranea* F. v. Muell.	Australia, N. Queensland	$n = 13$, $2n = 26$	Ehrendorfer et al. (1968)
T. piperita (Hook f.) Miers	*Drimys* sect. *Tasmannia*, "one polymorphic New Guinea species"	Papua-New Guinea, Wau	$n = 13$	Ehrendorfer et al. (1968)

Table 1. Continued.

Taxon	Published name	Provenance	$n/2n$	Reference
T. piperita (Hook f.) Miers	*Drimys piperita* Hook f.	Papua-New Guinea (two individuals)	a: $n = 13$, b: $2n = 52^{3}$	Ratter & Milne (1973)
T. purpurascens (Vickery) A. C. Smith	*Drimys purpurascens* Vickery	Australia, N. S. Wales	$n = 13$	Hotchkiss (1955)
T. stipitata (Vickery) A. C. Smith	*Drimys stipitata* Vickery	Australia, N. S. Wales	$n = 13$	Hotchkiss (1955)
***Drimys** brasiliensis* Miers subsp. *brasiliensis*	same	Brazil, São Paulo	$2n =$ ca. 86	Ehrendorfer, Silberbauer-Gottsberger & Gottsberger (1979)
D. brasiliensis Miers subsp. *sylvatica* (St. Hil.) Ehrend. & Gottsb.	same	Brazil, Rio de Janeiro	$2n = 86$	Morawetz (1984)
D. confertifolia Phil.	same	Juan Fernández Isl.	$n =$ ca. 43	Sun et al. (1990)
D. granadensis L.f. var. *mexicana* A. C. Sm.	same	Costa Rica	$n = \pm 43$, $2n = \pm 86$	Ehrendorfer et al. (1968)
D. winteri J. R. & G. Forst.[4]	same	cult.	$n =$ ca. 36^{5}, $2n =$ ca. 72^{5}	Strasburger (1905)
D. winteri J. R. & G. Forst.[4]	same	cult.	$2n = \pm 76^{5}$	Whitaker (1933)
D. winteri J. R. & G. Forst.[4]	same	cult. (two prov.)	$n = 43^{6}$	Raven & Kyhos (1965)
D. winteri J. R. & G. Forst.[4]	same	cult.	$n = 43$	Ratter & Milne (1973)
***Pseudowintera** axillaris* (J. R. & G. Forst.) Dandy	same	New Zealand, Wellington	$n = 43$	Hair in Raven & Kyhos (1965), Hair & Beuzenberg (1966)
P. colorata (Raoul) Dandy	same	New Zealand, cult.	$n = 43$	Hair in Raven & Kyhos (1965), Hair & Beuzenberg (1966)
P. traversii (Buchanan) Dandy	same	New Zealand, NW Nelson	$n = 43$	Hair & Beuzenberg (1966)
***Bubbia**[7] semecarpoides* (F. v. Muell.) Burtt	same	Australia, N. Queensland (two populations)	a: $2n = 86$ (–88) b: $2n = \pm 86$	Ehrendorfer et al. (1968)
B. spec.	same	Papua-New Guinea, Wau	$2n =$ (84)–86	Ehrendorfer et al. (1968)
***Belliolum**[7] crassifolium* (Baill.) v. Tiegh.	same	New Caledonia	$2n =$ ca. 86	Ehrendorfer et al. (1968)
***Zygogynum**[7] baillonii* v. Tiegh. (vel aff.)	same	New Caledonia	$n = 86$, $2n = 172$	Ehrendorfer et al. (1968)
Z. balansae v. Tiegh	*Zygogynum pomiferum* Baill. subsp. *balansae* (v. Tiegh.) Vink	New Caledonia	$n =$ ca. 43	Carr & Mc Pherson (1986)

Table 1. Continued.

Taxon	Published name	Provenance	$n/2n$	Reference
Canellaceae				
***Canella** winteriana* (L.) Gaertn.	*Canella alba* Murr.	USA, Florida	$2n = 28$	Goldblatt in Raven (1975), Goldblatt (1977)
***Cinnamodendron** axillare* (Nees & Mart.) Endl.	same	Brazil, Rio de Janeiro	$2n = 22$	Occhioni (1948)
***Capsicodendron** dinisii* (Schw.) Occhioni[8]	*Capsicodendron Dinissi*	Brazil, cult.	$2n = 26$	Occhioni (1945)

[1] Obviously erroneous for $2n = 26$ (see Raven & Kyhos, 1965).

[2] Possibly a triploid plant (Raven & Kyhos, 1965) or rather a wrong determination; no voucher!

[3] Obviously an autotetraploid plant.

[4] In view of the former wide circumscription of *Drimys winteri* (now limited to populations from central and southern Chile and adjacent Argentina), the exact determination of cultivated material remains doubtful

[5] Obviously erroneous for $n = 43$ or $2n = 86$, respectively.

[6] Partly with one additional fragment.

[7] The genera *Bubbia, Belliolum, Exospermum,* and *Zygogynum* s. str. are united by Vink (1985, 1993) under *Zygogynum* sensu lato.

[8] To be incorporated into *Cinnamodendron* (Kubitzki, 1993)?

Chromosome preparation by the air-drying method was performed according to Geber and Schweizer (1988), except that the root apices were softened in an enzymatic solution (2% cellulase plus 20% pectinase) at 37°C for 3 hours. The slides were stained in a DAPI (4′-6-diamidino -2 phenylindole) solution of 2 μg/ml in Mc Ilvain's buffer pH7 for 20 minutes and mounted in Mc Ilvain's buffer pH7 plus glycerol 2:1 (see Schweizer & Ambros, 1994). Chromosome fluorescence was observed with a Leizt Orthoplan fluorescence microscope with filter block A for DAPI epiluminescence. Microphotographs were taken on Kodak TMAX 400 film.

RESULTS

Well spread earlier and later mitoses and interphase nuclei from pretreated root tips of *Takhtajania perrieri* are shown in Figure 1. The nuclei belong to the prochromosome type: Their intensively stained elements correspond to the proximal and evidently heterochromatic segments of the chromosomes, which remain ± condensed during interphase and form about 36 larger and smaller chromocenters (Fig. 1b, c). These chromocenters exhibit a certain tendency toward agglomeration (particularly obvious in those of larger size) and are embedded in a very weakly stained granular-fibrous matrix. During mitotic prophase and early metaphase condensation of chromosome segments in a proximal → distal direction becomes apparent with the distal regions first visible as fibrous attachments (Fig. 1a). This process is concluded toward the end of metaphase (Fig. 1b), just before the onset of anaphase.

From the careful inspection of about 20 mitotic plates the somatic chromosome number $2n = 36$ can be ascertained. These chromosomes are of quite different sizes, the largest ones (about 4 to 5 pairs) reach a length of 2.0–2.5 μm in our pretreated material, the smallest (one pair), not always easily recognizable (see arrow heads in Fig. 1a and b), about 0.8 μm. We are not yet able to present a karyogram for *Takhtajania,* but there are certainly not four structurally ± identical chromosome sets and therefore no clear karyological indications for tetraploidy.

DISCUSSION

The interphase nuclei, chromosome size, and mitotic condensation pattern found in *Takhtajania perrieri* correspond well with the prochromosome type described by Tanaka (1971), Okada (1975: type C), and Morawetz (1986a: Gyrocarpus type) for many Magnoliid orders such as Magnoliales (Magnoliaceae, Annonaceae, etc.) and Laurales (Lauraceae, Calycanthaceae, etc.). Within Winteraceae and Win-

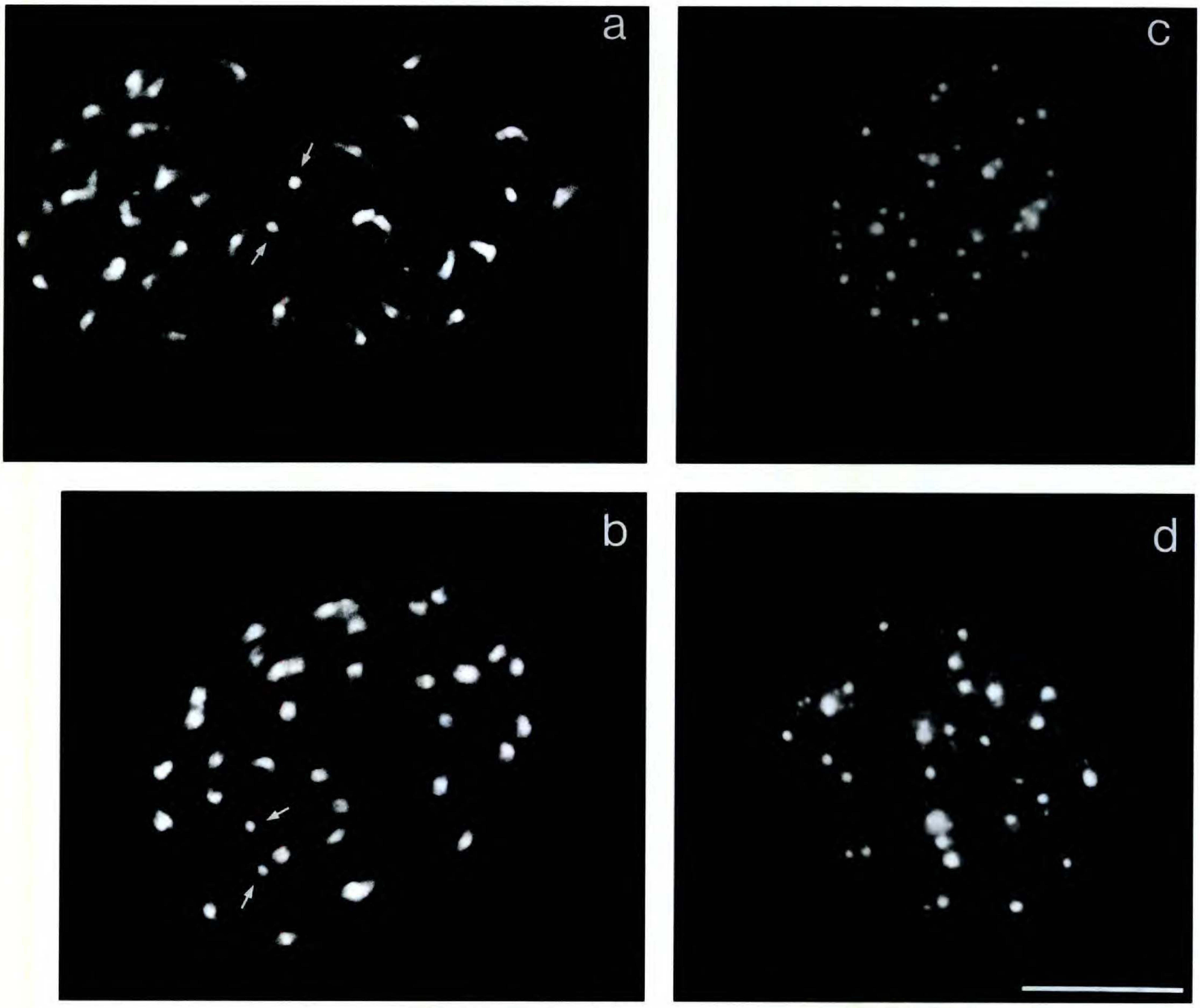

Figure 1. DAPI stained chromosomes (a, b) and interphase nuclei (c, d) of *Takhtajania perrieri* from pretreated root tips. In early metaphase (a) chromosomes fully condensed only in proximal segments, distally still fibrous, in late metaphase (b) completely condensed. Arrows mark the pair of smallest chromosomes. Interphase nuclei (c, d) are of the prochromosome type. The bar corresponds to 10 μm.

terales comparable karyological data are still limited, but *Drimys* s. str., in spite of its much higher chromosome number, exhibits quite similar karyological features to *Takhtajania* (Morawetz, 1984). Thus, there is no inconsistency in this respect to the placement of *Takhtajania* into the Winteraceae, and of Winterales, together with Magnoliales and Laurales, into the Magnoliid assemblage. Nevertheless, these karyological features may vary quite considerably within orders and families (e.g., *Cassytha* within Laurales: Okada, 1975; *Uvariopsis* and *Tetrameranthus* within Annonaceae: Morawetz, 1986a, b, 1988). This also applies to Winteraceae because preliminary and unpublished observations indicate that *Tasmannia* not only deviates from all other members of the family by its low chromosome number ($x = 13$), but also by much larger chromosomes and interphase nuclei that correspond rather to type B of Okada (1975). Together with the molecular evidence (Suh et al., 1993; Karol et al., 2000 this issue; Fig. 2) this argues strongly against the combining of *Tasmannia* and *Drimys* into a single genus as advocated by Vink (1970, 1988, 1993). Nevertheless, much more detailed and comparative studies on karyotypes, banding patterns, and DNA quantities in the members of Winterales and other Magnoliids are necessary before we can better understand the karyoevolution of these basal Angiosperms.

What are the phylogenetic implications of the chromosome number $2n = 36$ established for *Takhtajania* relative to other members of Winterales? The main evidence compiled in Table 1 is compared in Figure 2 with our present knowledge about the evolutionary divergence of the order. This shows that from four extant clades three (1–3) have remained on a low ploidy level: (1) *Takhtajania* ($n = 18$) and (2) *Tasmannia* ($n = 13$) within Winteraceae, and (3) Canellaceae ($n = 14, 13, 11$). Clade

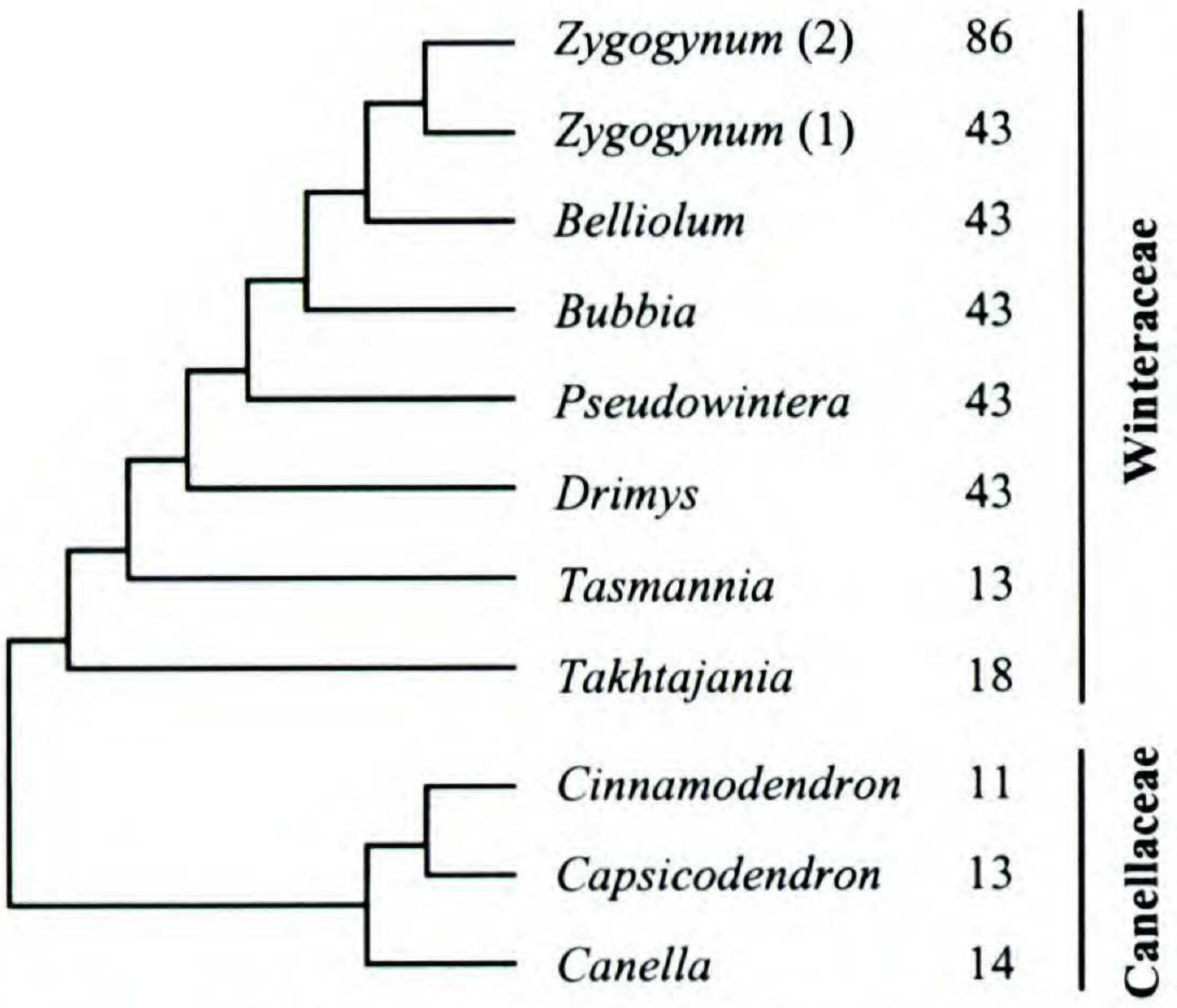

Figure 2. Molecular tree (ITS and *trn*L) of Winteraceae and Canellaceae, adapted from Karol et al. (2000) with genera for which chromosome numbers are known; haploid numbers (*n*) inserted. *Zygogynum* (1) = *Z. baillonii*, (2) = *Z. balansae*. See Table 1.

(4) has become stabilized on a higher polyploid level (n = 43, rarely with additional polyploidy: n = 86) and includes all remaining Winteraceae, i.e., *Drimys*, *Pseudowintera*, and the *Zygogynum* s.l. group of closely related genera. It is obvious that considerable evolutionary changes and numerous extinction events during the well documented and extremely long geological history of Winterales (since the Lower Cretaceous: see Doyle, 2000 this issue) must have resulted in the present-day highly relictual pattern of the Winterales.

There is some evidence that polyploids have become diploidized by considerable restructuring of their karyotypes (*Drimys*: Morawetz, 1984; *Takhtajania*: see above) and now appear as "paleopolyploids." In addition, chromosomal rearrangements must have led to dysploid and/or Robertsonian changes of base numbers at different ploidy levels. In Annonaceae and Eupomatiaceae this pattern of chromosomal evolution is still better preserved and more easily interpreted (Morawetz 1986a: fig. 16; 1988). It exhibits dysploid differentiation on the diploid level with extant taxa on $n = 7 \leftarrow 8 \rightarrow 9 \rightarrow 10$ as well as 4*x*, 6*x*, and higher polyploid series on each of these base numbers. This pattern can serve as a model for the evolutionarily much more "eroded" Winterales where such a reconstruction suffers from many extinction gaps. Nevertheless, Figure 3 illustrates possible links between chromosome numbers of extant representatives (Grant, 1982).

According to the ITS and *trn*L tree (Suh et al., 1993; Karol et al., 2000), *Takhtajania* with n = 18 and *Canella* with n = 14 are the most basal branches of Winteraceae and Canellaceae, respectively. If paleotetraploidy is postulated for these two extant relicts, then n = 13 in *Tasmannia* (Winteraceae) as well as n = 13 and n = 11 in the remaining Canellaceae could have originated by descending dysploidy. The origin of the highly polyploid Winteraceae clade (4) with n = 43 (paleo-12*x*?) was evidently linked with a remarkable duplication event in the ITS region (Suh et al., 1993; Karol et al., 2000). The present diversity center of this clade is in Australasia, i.e., former eastern Gondwanaland, from where it could have reached New Zealand with *Pseudowintera* and also South America via Antarctica (?) with *Drimys*.

In retrospect, Winterales correspond in their evolutionary pattern to other tropical, woody and "basal" Angiosperm groups (Ehrendorfer, 1976, 1987, 1995). They exhibit features of stasigenesis (evolutionary "erosion," much extinction), but also show signs of very active and recent eco-geographical radiation (e.g., in *Drimys*: Ehrendorfer et al., 1979; *Tasmannia*: Vink, 1970).

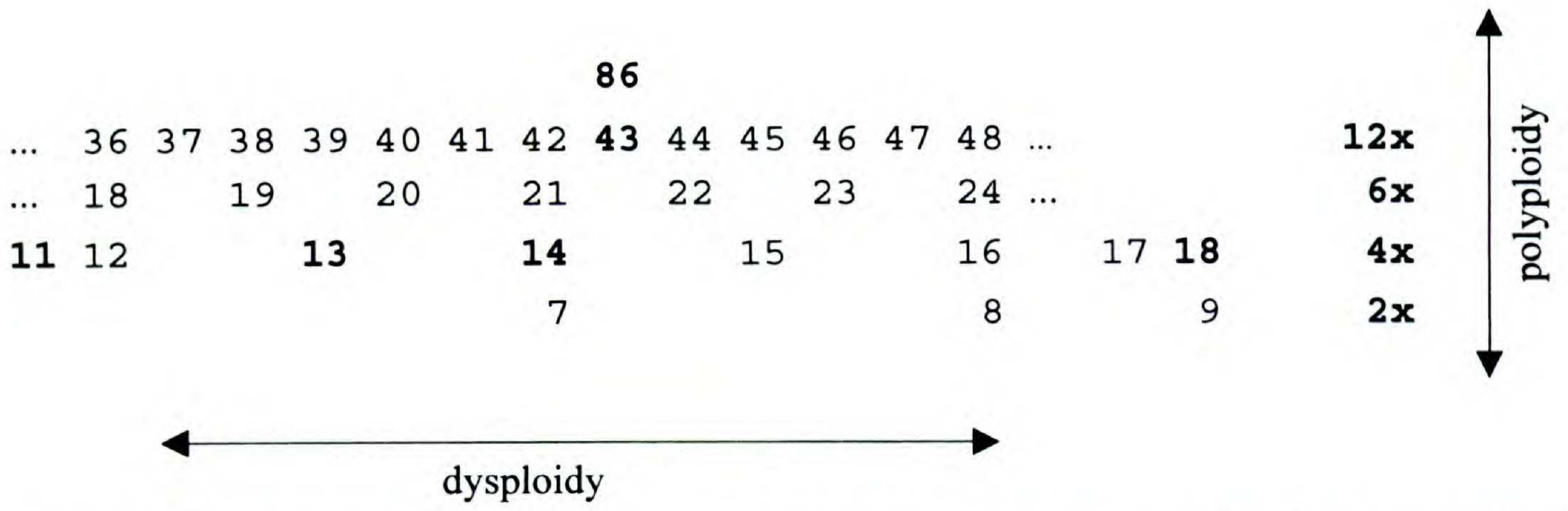
86
... 36 37 38 39 40 41 42 43 44 45 46 47 48 ... 12x
... 18 19 20 21 22 23 24 ... 6x
11 12 13 14 15 16 17 18 4x
7 8 9 2x
polyploidy
dysploidy

Figure 3. Possible origins of haploid chromosome numbers (*n*) of extant Winteraceae and Canellaceae (in bold print: 11, 13, 14, 18, 43, 86) as a result of polyploid and dysploid changes in hypothetical ancestral taxa (chromosome numbers in regular print).

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